

We Claim:

1. Novel thermostable, organic solvent resistant and high pH tolerant lipase gene variants having SEQ ID No. 2 of molecular wt 19443, SEQ ID No. 3 of molecular wt 19515 SEQ ID No. 4 of molecular wt 19456.9, SEQ ID No.5 of molecular wt.19487and SEQ ID No.6 of molecular wt. 19470.9
2. Novel gene variants as claimed in claim 1, wherein said gene variants are thermostable in the temperature range of about 45 to 95°C.
3. Novel gene variants as claimed in claim 2, wherein said gene variants are highly thermostable at the temperature in the range of about 55 to 90°C.
4. Novel gene variants as claimed in claim 1, wherein $T_{1/2}$ value is in the range of 6 to 685.
5. Novel gene variants as claimed in claim 1, wherein $T_{1/2}$ value is in the range of 7 to 677.
6. Novel gene variants as claimed in claim 1, wherein K_m value is in the range of 0.50 to 2.5 mM.
7. Novel gene variants as claimed in claim 1, wherein K_m value is in the range of 0.63 to 1.96 mM.
8. Novel gene variants as claimed in claim 1, wherein k_{cat} value is in the range of 4.5×10^{-2} to $8.5 \times 10^{-2} \text{ min}^{-1}$.
9. Novel gene variants as claimed in claim 1, wherein k_{cat} value is in the range of 5×10^{-2} to $8.1 \times 10^{-2} \text{ min}^{-1}$.
10. Novel gene variants as claimed in claim 1, wherein k_{cat}/K_m value is in the range of 4×10^{-2} to $10 \times 10^{-2} \text{ min}^{-1}$.
11. Novel gene variants as claimed in claim 1, wherein k_{cat}/K_m value is in the range of 4.1×10^{-2} to $9.7 \times 10^{-2} \text{ min}^{-1}$.
12. Novel gene variants as claimed in claim 1, wherein said gene variants are resistant to organic solvents selected from group of acetonitrile, isopropanol, dimethyl sulfoxide and dimethyl formide.
13. Novel gene variants as claimed in claim 4, wherein organic solvent used is acetonitrile.

14. Novel gene variants as claimed in claim 1, wherein residual activity of the gene variants is in the range of 25 to 100 % in presence of acetonitrile.
15. Novel gene variants as claimed in claim 1, wherein residual activity of the gene variants is in the range of 28.7 to 85.5% in presence of acetonitrile
- 5 16. Novel gene variants as claimed in claim 1, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and compounds thereof.
- 10 17. Novel gene variants as claimed in claim 16, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and compounds thereof.
- 15 18. An expression system for novel thermostable, organic solvent resistant and high pH tolerant lipase gene variants said expression system comprising of having SEQ ID No. 2 of molecular wt 19443, SEQ ID No. 3 of molecular wt 19515, SEQ ID No. 4 of molecular wt 19456.9, SEQ ID No.5 of molecular wt. 19487 and SEQ ID No.6 of molecular wt 19470.9 present in the vector pJO290.
19. An expression system as claimed in claim in 18, wherein said gene variants are thermostable in the temperature range of about 45 to 95°C.
- 20 20. An expression system as claimed in claim in 19, wherein said gene variants are highly thermostable at the temperature of about 55 to 90°C.
21. An expression system as claimed in claim in 18, wherein $T_{1/2}$ value is in the range of 6 to 685.
22. An expression system as claimed in claim in 21, wherein $T_{1/2}$ value is in the range of 7 to 677.
- 25 23. An expression system as claimed in claim in 18, wherein K_m value is in the range of 0.50 to 2.5 mM.
24. An expression system as claimed in claim in 23, wherein K_m value is in the range of 0.63 to 1.96 mM.
- 30 25. An expression system as claimed in claim in 18, wherein k_{cat} value is in the range of 4.5×10^{-2} to $8.5 \times 10^{-2} \text{ min}^{-1}$.

26. An expression system as claimed in claim in 25, wherein k_{cat} value is in the range of 5×10^{-2} to $8.1 \times 10^{-2} \text{ min}^{-1}$.
27. An expression system as claimed in claim in 18, wherein k_{cat}/K_m value is in the range of 4×10^{-2} to $10 \times 10^{-2} \text{ min}^{-1}$.
- 5 28. An expression system as claimed in claim in 27, wherein k_{cat}/K_m value is in the range of 4.1×10^{-2} to $9.7 \times 10^{-2} \text{ min}^{-1}$.
29. An expression system as claimed in claim in 18, wherein said gene variants are resistant to organic solvents selected from group of acetonitrile, isopropanol, dimethyl sulfoxide and dimethyl formide.
- 10 30. An expression system as claimed in claim in 29, wherein organic solvent used in acetonitrile.
31. Novel gene variants as claimed in claim 18, wherein residual activity of the gene variants is in the range of 25 to 100 % in presence of acetonitrile.
32. Novel gene variants as claimed in claim 31, wherein residual activity of the gene variants is in the range of 28.7 to 85.5% in presence of acetonitrile
- 15 33. Novel gene variants as claimed in claim 18, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and compounds thereof.
- 20 34. Novel gene variants as claimed in claim 33, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and compounds thereof.
- 25 35. A method of preparing an expression system of novel thermostable, organic solvent resistant and high pH tolerant lipase gene variants having SEQ ID No. 2 of molecular wt 19443, SEQ ID No. 3 of molecular wt 19515, SEQ ID No. 4 of molecular wt 19456.9, SEQ ID No.5 of molecular wt. 19487 and SEQ ID No.6 of molecular wt 19470.9 said method comprising the steps of :
- 30 (a) isolating and purifying lipase gene from *Bacillus subtilis*, ,
- (b) cloning lipase gene isolated in step (a) in vector pJO290,

- (c) generating gene variants from lipase gene isolated in step (a) by random mutagenesis and site-directed mutagenesis using forward primer JOF having SEQ ID No.13 and reverse primer JOR having SEQ ID No. 14,
- (d) cloning the gene variants obtained in step (c) in plasmid vector pJO290, and
- (e) ligating the cloned gene variants of step (d) in *E.coli* JM109.
36. A method as claimed in claim 35, wherein said gene variants are thermostable in the temperature range of about 45 to 95°C.
37. A method as claimed in claim 36, wherein said gene variants are highly thermostable in the temperature range of about 55 to 90°C.
38. A method as claimed in claim 35, wherein $T_{1/2}$ value is in the range of 6 to 685.
39. A method as claimed in claim 38, wherein $T_{1/2}$ value is in the range of 7 to 677.
40. A method as claimed in claim 35, wherein K_m value is in the range of 0.50 to 2.5 mM.
41. A method as claimed in claim 40, wherein K_m value is in the range of 0.63 to 1.96 mM.
42. A method as claimed in claim 35, wherein k_{cat} value is in the range of 4.5×10^{-2} to $8.5 \times 10^{-2} \text{ min}^{-1}$.
43. A method as claimed in claim 42, wherein k_{cat} value is in the range of 5×10^{-2} to $8.1 \times 10^{-2} \text{ min}^{-1}$.
44. A method as claimed in claim 35, wherein k_{cat}/K_m value is in the range of 4×10^{-2} to $10 \times 10^{-2} \text{ min}^{-1}$.
45. A method as claimed in claim 44, wherein k_{cat}/K_m value is in the range of 4.1×10^{-2} to $9.7 \times 10^{-2} \text{ min}^{-1}$.
46. A method as claimed in claim 35, wherein said gene variants are resistant to organic solvents selected from group of acetonitrile, isopropanol, dimethyl sulfoxide and dimethyl formide.
47. A method as claimed in claim 46, wherein organic solvent used in acetonitrile.
48. A method as claimed in claim 35, wherein residual activity of the gene variants is in the range of 25 to 100 % in presence of acetonitrile.

49. A method as claimed in claim 48, wherein residual activity of the gene variants is in the range of 28.7 to 85.5% in presence of acetonitrile
50. A method as claimed in claim 35, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand
- 5 51. damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and compounds thereof.
52. A method as claimed in claim 50, wherein the gene variants have inherent ability to withstand high pH in the range of 9 to 13; ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates,
- 10 proteases and compounds thereof.